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13. ABSTRACT *(Maximum 200 Words)*

Nucleosomes are folded together into chromatin structures that inhibit transcription. Studies here test the idea that an enzyme, Gcn5, that regulates chromatin folding is important for p53 functions and for estrogen responses. Experiments were proposed to 1) determine whether Gcn5 serves as a coactivator for activation of gene expression by the estrogen receptor 2) examine biochemical, molecular, and genetic connections between Gcn5 and p53 and 3) to generate a mammary gland specific 'knock out' of Gcn5 in mice to create a mouse model for Gcn5 functions in breast development and tumor formation. We have made good progress towards all three aims. We have examined Gcn5-dependence of estrogen responses in ER+ cell lines. We have generated mice that carry null alleles for both Gcn5 and p53 in cis on chromosome 11. We have generated mice that carry a conditional disruption allele for Gcn5 that will allow us to do the breast-specific knock out in the coming year. Our studies will provide new information about breast cancer biology. Moreover, they will allow us to determine whether histone acetyltransferases might provide targets for development of new drug therapies or diagnostic agents, furthering our advancement towards eradication of this disease.

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Table of Contents

INTRODUCTION:

The compaction of DNA into chromatin in the eukaryotic nucleus limits transcription by limiting access of the basal transcription machinery and regulatory proteins to their target sequences (Edmondson and Roth, 1996). Chromatin remodeling is now recognized as a central feature of gene regulation. Two major classes of chromatin remodeling activities have been identified to date (Struhl, 1998; Wade and Wolffe, 1999). One class includes large ATP-driven complexes typified by Swi/Snf, which regulate nucleosome placement and movement. The second class is comprised of enzymes responsible for regulating post-translational modifications of the histone proteins. Of these, histone acetyltransferases (HATs) and histone deacetylases (HDACs) are best characterized (Roth et al., 2001). Particular HATs, such as AIB1, have been implicated in cancers, including breast cancer (Anzick et al., 1997). Other HATs are required as cofactors for tumor suppressor activities such as p53 or for hormonal responses (Blanco et al., 1998; Liu et al., 1999; Schiltz and Nakatani, 2000). The GCN5 HAT is highly related to PCAF (Xu et al., 1998), which has been linked both to p53 and estrogen receptor functions. Experiments supported by this grant are designed to determine the role of GCN5 in these processes and to determine whether GCN5 serves as a suppressor or enhancer of breast tumor formation. The importance of GCN5 to normal breast development will be determined as well. The proposal has three specific aims: 1) to determine whether GCN5 serves as a coactivator for the estrogen receptor 2) to examine biochemical, molecular and genetic connections between GCN5 and p53 and 3) to generate a mammary gland specific 'knock out' of GCN5 in mice. The results of these studies may provide new targets for therapies or novel diagnostic tools.

BODY:

Our Statement of Work was organized into three main tasks. We have made good progress on each of these tasks in this first two years of funding, as described below:

Task1: To determine whether GCN5 serves as a coactivator for the estrogen receptor in transcriptional activation in mammalian cells.

- Goal 1: Transfect NIH 3T3 cells with expression constructs for the estrogen receptor (ER), FLAG-tagged GCN5, and an estrogen responsive reporter gene. Monitor expression of the reporter gene in the presence and absence of estradiol.

  Progress: Completed. Unfortunately, the results of these transient transfection experiments were inconclusive and suggested to us that we needed to create stable cell lines carrying estrogen responsive reporter genes integrated into the chromosome and that we needed to examine additional types of cells. To this end, we selected stable cell lines (MCF7 and CV1 cells) that carry an integrated reporter gene and but these cells were unhealthy and were not useful for our studies. Therefore, we transfected GCN5 expression constructs into breast cancer cell lines that express the ER. GCN5 protein was not stable in these cells, again thwarting our efforts to examine its role in estrogen responses. Despite our best efforts, this goal may not be feasible, but we may continue to explore additional cell lines. However, the mouse experiments described in Task 3 will be more definitive than these cell culture experiments, so priority will now be shifted towards that task.

- Goal 2: Construct mutated forms of GCN5 for transfection experiments to determine the domains required for estrogen mediated functions.

  Progress: We have constructed point mutations in the GCN5 HAT catalytic center. Although we originally planned to test these in cell culture experiments, we have now decided to test them in mice, given the problems encountered above. To this end, we have created targeting alleles that will

replace the wild type Gcn5 gene with these mutated alleles and are now introducing these into embryonic stem cells, for the subsequent generation of mutant mice.

- Goal 3: Perform in vitro binding experiments to determine if GCN5 interacts directly with the estrogen receptor.

  Progress: These experiments are still on hold until (or unless) we demonstrate some role for Gcn5 in estrogen responses in the above experiments.

- Goal 4: Perform co-immunoprecipitation experiments to determine if GCN5 is associated (directly or indirectly) with the estrogen receptor in vivo.

  Progress: On hold pending the outcome of the above experiments.

Task 2: To examine the role of GCN5 in p53 functions.

- Goal 1: Express and purify recombinant p53.

  Progress: Completed.

- Goal 2: Test recombinant p53 as a substrate for recombinant GCN5.

  Progress: Completed. We found that GCN5 can acetylate p53 in vitro (Fig. 1) and are currently determining which domains in p53 are acetylated.

- Goal 3: Perform co-transfection experiments to determine if GCN5 augments p53 transactivation in vivo, and if so, determine which domains in GCN5 are required for this effect.

  Progress: Completed. Unfortunately, we could never demonstrate any augmentation of p53 responses upon cotransfection with GCN5 in transient transfection experiments (see Fig. 2 for example). This lack of response may indicate that GCN5 functions may only be required when the reporter gene is packaged into chromatin. Therefore we examined expression of the endogenous p21 gene, which is p53 responsive. Again we saw no effect of addition of GCN5 (data not shown). Since we do not have any cell lines that lack enodgenous GCN5, it may be that endogenous levels of this enzyme are obscuring any effects of the transfected GCN5. Therefore we are hoping to create a 'dominant negative' form of GCN5 in the coming year.

- Goal 4: Cross mice heterozygous for the GCN5 null allele with mice homozygous for the p53 allele.

  Progress: Completed. We have successfully generated mice that are null for p53 and heterozygous for the GCN5 null allele. We are continuing our breeding to generate more of these mice, as well as mice that heterozygous for both the p53 null allele and the Gcn5 null allele.

- Goal 5: Cross offspring from the above matings to generate mice that are null for both p53 and GCN5, to see if loss of p53 rescues the embryonic lethality resulting from GCN5 loss.

  Progress: In progress and on track for completion in year 3. Gcn5 and p53 are both encoded on chromosome 11, so we had to isolate mice that had undergone a recombination event to palce both mull alleles on the same copy of this chromosome. These are now in hand.

Task 3: To generate a mammary gland-specific 'knock out' of GCN5 in mice.

- Goal 1: Construct targeting vector for GCN5 gene replacement.

  Progress: Completed.

- Goal 2: Transform targeting vector in ES cells and select/screen properly targeted cells.

  Progress: Completed. We identified 7 independent clones that carry the properly targeted replacement allele (Fig. 3, for example).

- Goal 3: Inject above ES cells into blastocysts to generate chimeric mice.

  Progress: Completed.

- Goal 4: Breed chimeric mice with wild type mice to generate mice heterozygous for the replacement allele.

  Progress: Completed.

- Goal 5: Intercross heterozygous mice to generate mice homozygous for the replacement allele.

  Progress: In progress. We will also cross the heterozygotes with mice heterozygous for our original null allele to generate mice that carry one floxGcn5 allele and one null allele.

- Goal 6: Breed homozygous mice with WAP-cre transgenic mice.

  Progress: On track for completion during year 3.

- Goal 7: Analyze GCN5 removal and mammary gland development and mammary tumor formation in female mice resulting from the above cross before, during, and after pregnancy.

  Progress: On track for completion during year 3 of the grant.

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstration that GCN5 can acetylate p53 in vitro.
- Creation of mice that carry null alleles of p53 and Gcn5 in cis on chromosome 11.
- Construction of a floxed allele of GCN5 and creation of mice that carry this allele.

REPORTABLE OUTCOMES:

In first two years of this grant, we have made good progress towards our goals, but our work is not yet at a suitable stage for publication. We anticipate publications in the next year.

CONCLUSIONS:

We have determined that transient transfection experiments are limited in their ability to delineate the role of GCN5 as a coactivator in p53 or ER responses. Therefore we will focus on the mouse model created in Task 3. We have also determined that Gcn5 protein stability is altered in certain cell lines. Whereas this is an interesting finding, follow up studies to determine the molecular basis of this difference is beyond the scope of this grant. Our studies may reveal new ways of diagnosing breast tumors or new strategies for treatment. In addition, our last task will provide novel insights into the role of chromatin modifying activities in normal breast development as well as in tumor formation.

REFERENCES:

Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. (1997). AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer, Science *277*, 965-8.

Blanco, J. C. G., Minucci, S., Lu, J., Yang, X.-J., Walker, K. K., Chen, H., Evans, R. M., Nakatani, Y., and Ozato, K. (1998). The histone acetylase PCAF is a nuclear receptor coactivator, Genes and Devel *12*, 1638-1651.

Edmondson, D. G., and Roth, S. Y. (1996). Chromatin and transcription, FASEB Journal *10*, 1173-82.

Liu, L., Scolnick, D. M., Trievel, R. C., Zhang, H. B., Marmorstein, R., Halazonetis, T. D., and Berger, S. L. (1999). p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage, Mol Cell Biol *19*, 1202-1209.

Roth, S. Y., Denu, J. M., and Allis, C. D. (2001). Histone Acetyltransferases, Annu Rev Biochem *70*, 81-120.

Schiltz, R. L., and Nakatani, Y. (2000). The PCAF acetylase complex as a potential tumor suppressor, Biochim Biophys Acta *1470*, M37-53.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms, Genes and Dev *12*, 599-606.

Wade, P. A., and Wolffe, A. P. (1999). Transcriptional regulation: SWItching circuitry, Curr Biol *9*, R221-4.

Xu, W., Edmondson, D. G., and Roth, S. Y. (1998). Mammalian GCN5 and P/CAF acetyltransferases share homologous amino-terminal domains important for the recognition of nucleosomal substrates, Mol Cell Biol *18*, 5659-5669.

APPENDIX

Figure 1. Targeting strategy for floxed GCN5 allele for creation of breast-specific knockout.

A. Diagrammed are the Gcn5 genomic locus, the targeting vector carrying loxP recombination sites flanking the Gcn5 coding region (floxed allele), and the predicted structure of the correctly targeted allele. B. Southern blots using the indicated 5' and 3' probes to demonstrate correct integration of the floxed Gcn5 allele in embryonic stem cells. The same Southern screens were used to identify mice carrying this allele in subsequent experiments (data not shown).